ENVIRONMENTAL AND GENETICAL VARIATIONS IN YIELD AND COLONY SIZE OF COMMERCIAL YEASTS[1]

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One essential requirement of a good commercial baker's yeast is the ability to transform the maximum amount of nutrients in the fermentor into yeast in the shortest possible time. Yeasts vary in this property, and our first step in an attempt at yeast improvement included the collection of various strains, and the design of adequate tests for selecting the high-yielding ones.

This yield test was designed primarily to reject inferior cultures. The early data are comparative and indicative rather than absolute. In handling hundreds of cultures in the laboratory it is not feasible to duplicate plant conditions, and yields of the same yeast are relatively lower in the test-tube than in the plant. Our ultimate objective is to devise a test which will predict accurately the behavior of a yeast under plant conditions.

MEDIA AND METHODS

1. The Yield Test.—Broth was made by mixing carbohydrate and protein mashes obtained from the Anheuser-Busch yeast plant, in proportions which produced a medium about one half as concentrated as that used in the fermentors. This resulted in a medium containing 0.8 per cent sucrose and 0.7 per cent nitrogen-containing solids. It was neutralized by adding an excess of powdered calcium carbonate. After autoclaving for almost an hour to remove the heat-coagulable proteins, it was filtered with diatomaceous earth. A medium resembling that used commercially was designed to avoid the selection of strains of yeast not adapted to the ordinary commercial medium. Twenty cc. were placed in an 8 x 1-inch test-tube. This test-tube had previously received a small inverted gas tube, the lower opening of which was cut off at a slant to prevent a seal forming by contact with the base of the larger tube. After the tubes were autoclaved, approximately the same amount of inoculum was introduced into each tube with a spatulate needle. Within wide limits the amount of inoculum seems to make little difference in the yield. Twenty-four hours later the depth of the column of gas in the inverted tube was read to the nearest half centimeter. After 48 hours the contents were shaken up and 10 cc. were poured into a Hopkins vaccine tube and centrifuged for 20 minutes at 2000 r.p.m. The volume of the yeast was read off directly and recorded in hundredths of a cc. per 10 cc. of broth.

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2. MDY Agar.—Our standard medium for plating yeasts is malt-dextrose-dried yeast agar of the following formula:

Malt extract	10.0%
Dextrose	.5%
Dried brewer's yeast	.5%
Agar	3.0%
$CaCO_3$	1.0%

3. CM Agar.—Carbohydrate protein mash agar was made simply by adding 3 per cent agar and 1 per cent $CaCO_3$ to the broth used in the yield test.

4. Pr Agar.—Prune agar was made by diluting the syrup from canned prunes with an equal volume of tap water and adding 1 per cent $CaCO_3$ and 3 per cent agar.

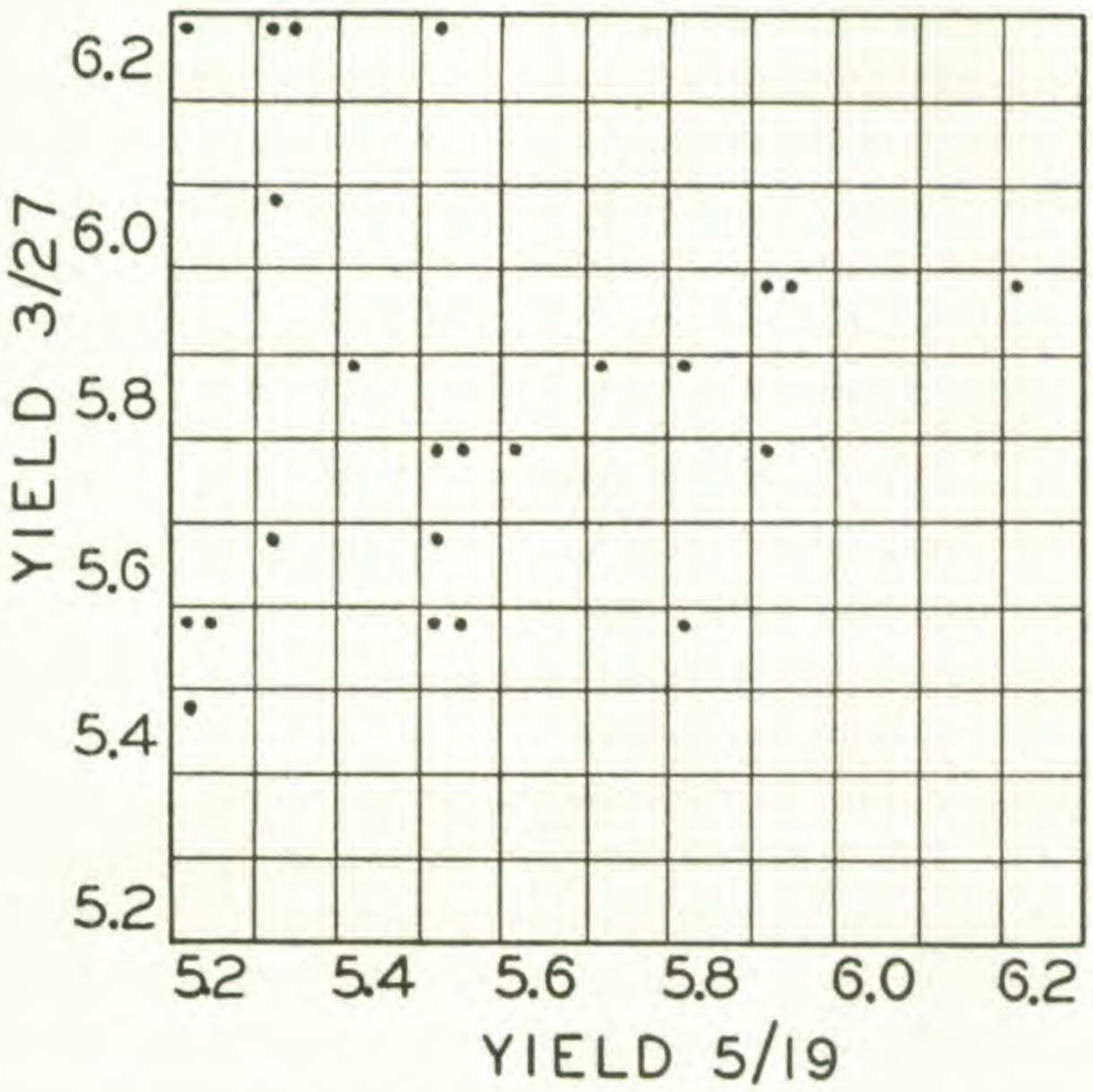


Fig. 1. Diagram showing correlation between yields of selected colony cultures in successive tests.

REPRODUCIBILITY OF DATA ON YIELD

A large number of duplicate tests with two different tubes of broth proved that the yields usually checked within .2 of a hundredth of a cc. when the tests were carried out at the same time. We have found on repeated trials that yeasts yielding less than 4.0 (hundredths of a cc.) usually remain in the low ranges and that those yielding 5.5 or more are not found in the lower brackets.

The data in fig. 1 show the yield of a group of different cultures tested on March 27, as compared with transfers from those cultures tested on May 19.

Although the correlation is not so high as might be desired, none of these relatively good yeasts was found on the second test to fall into the undesirable low-yielding category, i.e., in the ranges below 4.5. An interesting fact concerning the low-yielding yeasts is that the sediment in the centrifuge tube is usually much darker than that in the high-yielding ones.

PURIFICATION OF MIXTURES BY THE PLATING TECHNIQUE

Many yeasts are mixtures of genetically different kinds of cells which can be separated. The two principal techniques for effecting separation are (1) plating and (2) single-cell isolation. Cells are plated by spreading a loopful of a dilute suspension of the yeast over the surface of an agar plate. Individual colonies on the thinly seeded area of the plate usually originate from single cells. Single colony selection is more fruitful than single-cell isolation in separating mixtures, for it is possible to examine and compare several hundred colonies by spreading only a few plates. Colonies differing in shape and topography, selected from a plate and spread in turn on other plates, are generally found to repeat their respective characters, indicating that the original colonies were pure. The colonies must be rather well spaced if this technique is to be successful. The second method of single-cell isolation requires the selection of individual yeast cells with a micromanipulator. This is not at all difficult with yeast cells but plating yields so much more abundant results that it is the preferred method. In an unpublished study of variations of bacteria we made over 300 single-cell isolations of a bacterium with a relatively high mutation rate and found that plating gave results consistent with those obtained by single-cell isolations. Most bacteriologists studying variation agree with this conclusion.

HETEROGENEITY OF COMMERCIAL YEAST

Many commercial yeasts contain a heterogeneous mixture of biotypes, and this is especially true of old test-tube cultures. We recognize two general classes of colonies in these mixtures which we have called the primary and secondary types. The primary type usually seems to carry the desirable qualities. Various secondary genotypes are also present, but they are apparently deleterious rather than beneficial. Some of our data suggest that the commercial propagation of yeast considerably reduces the heterogeneity. The heterogeneity can be demonstrated by streaking on a rich substrate (such as MDY agar). The primary colonies from which the yeast derives its superior qualities are large, white, entire, smooth, hemispherical and opaque. The secondary colonies are generally much smaller than the primaries and are often brownish with lobed margins and rough surfaces (pl. 2, fig. a) or gray, translucent and flat (fig. b). Two typical small secondary colonies are also shown in pl. 3, fig. a. The secondary colonies often appear in a variety of types, indicating that they differ among themselves. The possibility that they may carry some valuable qualities seems unlikely for many

tests have revealed that these forms are extremely inferior in yield and fermentative ability. This view is further supported by the fact that most good commercial yeasts when fresh produce few secondary colonies. The secondary colonies sometimes have distinctive cell shapes. Rough colonies often have long, slender cells while smooth ones usually have ellipsoidal cells. However, there is much variation in cell size and shape in any colony. We have found a few extreme cases in which this character was very useful diagnostically.

AN EXAMPLE OF HETEROGENEITY IN A BAKERS' YEAST

A striking example of sharply bimodal heterogeneity in a bakers' yeast was discovered by plating a sample from a pound package of yeast. The colonies appearing in the plates shown in pl. 2, fig. b were of two kinds: typically large and opaque primary colonies, and flat, translucent, and gray secondary colonies. The cells of the primary colonies were of normal size, but those of the secondary colonies were generally small. Tests for yield revealed the following distribution:

Yield class range	*Primary colonies*	*Secondary colonies*
2.0 – 2.9	0	11
3.0 – 3.9	0	1
4.0 – 4.9	0	0
5.0 – 5.9	0	0
6.0 – 6.9	11	0
7.0 – 7.9	3	0

The two types of colonies appeared in about equal numbers on the plate, indicating that about half the package was made up of a distinctly inferior yeast. This is the only heterogeneous yeast which we have found containing two such sharply contrasted forms. More frequently a wide range of variation is encountered.

SELECTION WITHIN A CLONE

An attempt was made to determine if the primary colonies can be further subdivided into yeasts of high and low yield. The results are recorded graphically in fig. 2. On the horizontal scale are indicated the various class ranges in yield of hundredths cc. per 10 cc. of medium. On May 31 a commercial live dried yeast was plated on MDY agar and 16 large colonies selected and tested for yield. Thirteen colonies plated from the lowest-yielding culture (6.3) were tested on June 6a. On the same day (June 6b) 12 colonies originating from one of the highest-yielding colonies were tested, but no significant differences were revealed. This suggests that the primary colonies of the commercial yeast are all closely related genetically.

In any colony there are rather wide variations in cell size. Not unusually small colonies which produce poor yields contain an excess of small cells. An effort was made to determine if large and small cells from one colony produced high- and low-yielding cultures. On June 8, 6 single-cell cultures were made of

the large cells, and 4 single-cell cultures of small cells from the same high-yielding culture. No significant difference was found between these two groups, indicating that the variations in cell size are not necessarily indicative of genetical differences.

On June 10 tests a and b revealed that the highest- and lowest-yielding cultures from June 6a did not produce significantly different types. Also the c and d tests showed that the highest- and lowest-yielding colonies from June 6b did not result in differently yielding progenies. Finally, on June 15, 14 colonies selected from the highest-yielding colony in the June 10d test were found to be not significantly better than the original colonies.

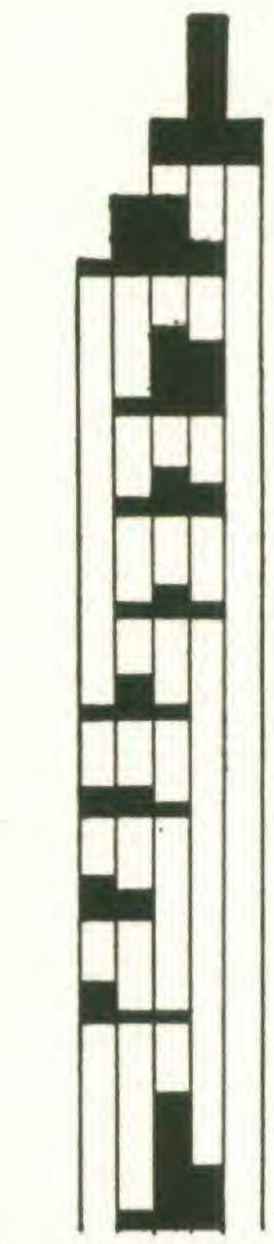
Throughout the record, it is clear that the day on which the tests were made, rather than the yield of the parent culture, affected the determination of the mode. The lowest-yielding group was obtained on June 10, the next on June 6, June 8, and June 15, and the highest-yielding group on May 31. Since the tests were made at room temperature, because of limitation of incubator space, it appears that if more accurate duplication were desired, it would be necessary to incubate the tubes. As has already been pointed out, the tests are only to eliminate inferior stocks, and while accurate temperature control is desirable it is not essential. Although temperature probably determines the position of the mode, the variation about the mode is apparently due to other local environmental conditions.

In the course of the experiment, 10 small secondary colonies were selected from various plates and tested along with the primary colonies. The two histograms (fig. 2b) show that most of these secondary colonies are distinctly inferior in yield. Secondaries were not present on all plates and when present usually made up less than 1 per cent of the total number.

In one series of selections the highest-yielding member of each set was successively chosen to produce the next culture generation. Selections were made serially from the following populations (a) May 31, (b) June 6b, (c) June 10d and (d) June 15. This intensive selection did not result in any improvement of the culture, indicating that the primary colonies all belong to one biotype.

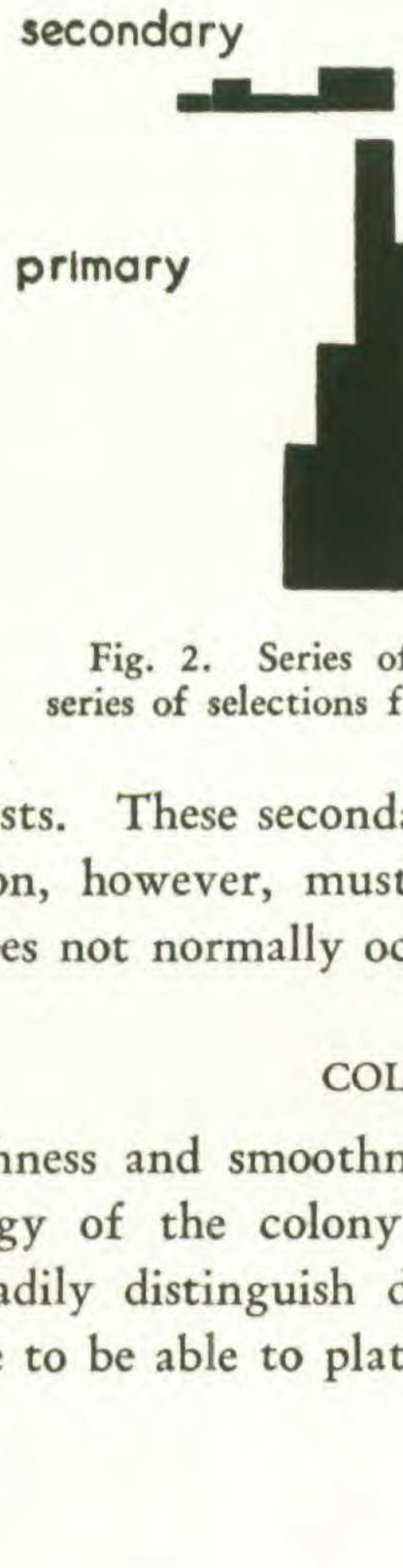
Variation due to environmental differences usually have a range of from 0.5 to 1.0 hundredths of a cc. in a 10-cc. sample at any given temperature of incubation. The use of class ranges with a magnitude of .5 and the fact that all the samples usually fell in three classes make the environmental variations look much larger than was actually the case. The few samples in the outer ranges usually fell close to the central class. With many yeasts we have found that a dozen or more samples from one plate may all fall within a range of .2 hundredths of a cc. The yeast used in this experiment showed greater variations in a given determination than were usually encountered. This difference in the ranges of variability of different clones unfortunately means that a genetically significant variation in one yeast may not necessarily be significant in a second yeast.

It is clear that the secondary colonies yielding less than 5.0 are distinctly in-



DATE	SOURCE CULTURE	YIELD
May 31	Commercial yeast	Undet.
June 6a	May 31	6.3
June 6b	May 31	7.0
June 8	May 31 large cells	7.2
June 8	May 31 small cells	7.2
June 10a	June 6a	5.4
June 10b	June 6a	6.8
June 10c	June 6b	5.8
June 10d	June 6b	6.8
June 15	June 10d	6.5

a. THE EFFECT OF SELECTION WITHIN A CLONE ON YIELD.



b. COMPARISON OF THE YIELDS OF PRIMARY AN SECONDARY COLONIES.

Fig. 2. Series of histograms showing the frequency of yield in a series of selections from a yeast clone. See text.

ferior yeasts. These secondary colonies probably arise by mutation or segregation. Segregation, however, must occur rather rarely since spore formation in these strains does not normally occur except under special conditions.

COLONY SIZE ON VARIOUS MEDIA

Roughness and smoothness or other characters affecting the topography and morphology of the colony are easily recognized as stable genetical characters which readily distinguish different strains or variants in yeasts. It is a great advantage to be able to plate out cultures on agar and observe the distribution of

these different types. Although all the colonies on an agar plate may be readily recognized as being either rough or smooth they are never all of the same size. When the colonies are plated thickly they are generally small, apparently because they interfere with each other. At the edge of the seeding, where only a few colonies appear, they are usually larger. This type of size variation is purely environmental and has no genetic basis. It can be seen very clearly in pl. 3, figs. a and e.

The true dwarf colonies, which we have called secondary colonies, can be detected merely by their small size if the test is made under the proper conditions. When colonies are planted thinly, these secondary colonies remain small when compared to the primaries. Since the secondary colonies have been found to yield considerably less than the primaries, it is an especial advantage to be able to form some opinion of the distribution of primary and secondary forms in a culture merely by inspection of the colonies on an agar plate.

On a poor medium the situation is quite different. At the first transfer to a poor medium there may be no correlation between size of colony and yield. Fewer colonies appear following equal inoculation on a poor agar than on a rich medium, and the colonies that do grow vary greatly in size. The fact that the number of colonies is reduced proves that only a small fraction of the cells survive transfer to the inferior medium. Plate 3, figs. a, b, and c show agar plates on which equal numbers of cells were plated on MDY agar (pl. 3, fig. a), on CM agar (pl. 3, fig. b) and on Pr agar (pl. 3, fig. c). On the latter two media only a few colonies appeared, and they varied greatly in size. Twenty-four colonies on a CM agar plate were graded according to size, "A" indicating the largest and "F" the smallest size class. They were tested for yield with the results shown below.

Yield class range	*Colony Size*					
	Largest					*Smallest*
	A	B	C	D	E	F
4.5 – 4.9		1				1
5.0 – 5.4	1		3	1	2	
5.5 – 5.9		1	1	4	2	3
6.0 – 6.4					3	1

The four highest yielding cultures were obtained from colonies falling in the two smallest size categories. These results show that the differences in colony size on a poor medium do not result from true genetic differences in vigor. Only a few cells survive the shock of transplantation, and the surviving cells which are able to produce colonies do not fully recover from the shock of the transfer even after the colonies originating from the survivors have attained their full growth. However, a second transfer to the same medium makes the adaptation complete as far as can be judged from colony size.

Figure 3 is a record of variations in size of colonies after serial plating on CM, MDY, and Pr agar. Large, medium, and small colonies selected from CM plates, like those shown in pl. 3 fig. b, were transferred to CM medium. All produced

uniformly large colonies following this second transfer to the identical inferior medium, corroborating the view that the size differences shown on the first CM plate were not due to genetic differences in vigor. Transfer to MDY medium also produced uniformly large colonies. However, transfer from CM to Pr agar did not result in uniformity, but the plates made from large and small colonies both showed considerable variation in colony size. When large colonies from the first Pr plate were transferred to a second Pr plate only uniform large colonies appeared. Moreover, when the small colonies from the first Pr plate were transferred to a second Pr plate uniform large colonies resulted. This proves that transfer from CM to Pr produces shock just as transfer from MDY to either Pr or CM did. After two transfers on a specific poor medium, adaptation to this specific poor medium occurs which makes the culture capable of producing uniformly large colonies on the inferior substrate.

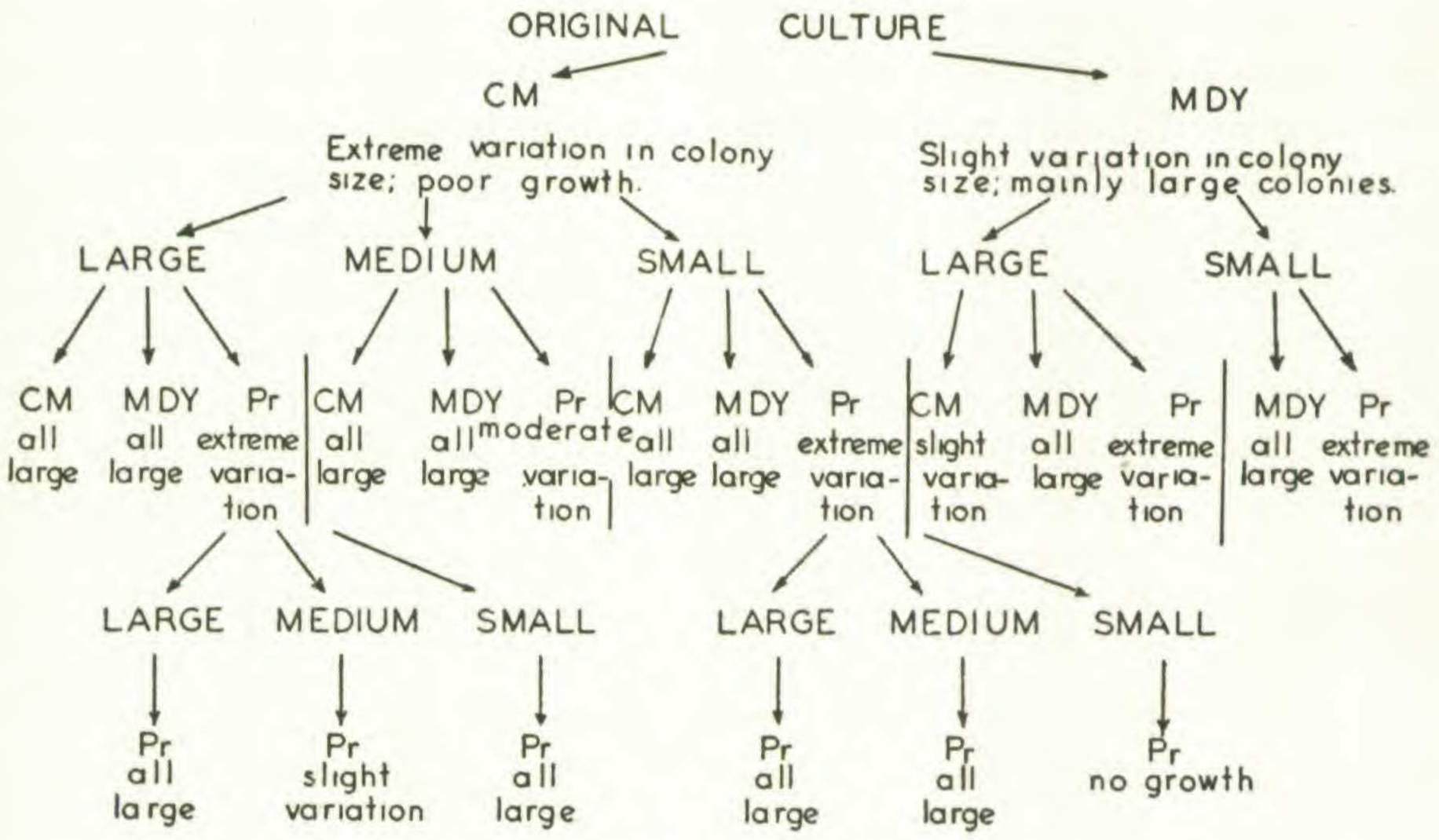


Fig. 3. Variations in colony size on MDY, CM, and Pr agars and the effects of selection of large and small colonies.

It is an especial advantage to be able to form some opinion of the distribution of primary and secondary forms in a culture simply by sowing an agar plate. However, primary and secondary colonies can only be distinguished when the culture is plated on a rich medium supplying all the necessary nutrients. Although one might expect that a medium lacking in certain essentials would be better adapted to making distinctions of this kind, on poor media there is so much reduction of size, probably due to the shock of the first transfer and from other causes, that the distinction fails. On the MDY medium two types are easily recognized. This does not imply that either the primary or secondary colonies are genetically uniform as a class. We have found in *Neurospora* that a good medium often con-

ceals minor genetical difference. The apparent difference between yeast colonies on a poor medium are artefacts.

These experiments prove that it is possible to distinguish high- from low-yielding yeasts by colony size provided a rich medium such as MDY is used. However, the potentially high-yielding cells do not necessarily produce the larger colonies when plated on inferior agar. One might expect that the differences between survivors after exposure to exceptionally adverse conditions would be due to true genetic differences, but it appears from these experiments that if the conditions are too severe the differences in colony size among the survivors is the result of a series of relatively unpredictable accidents and not indicative of hereditary vigor.

THE EFFECT OF GENETIC DIFFERENCES ON SURVIVAL UNDER ADVERSE CONDITIONS

Plate 2, figs. a, b, and c show the colonies appearing when the R strain of yeast is sown for the first time on MDY, CM, and Pr agar respectively. This R strain (R does not signify "Rough" but is merely a serial designation) is an exceptionally good baking yeast with a rather low yield but high baking strength. Subsequent analysis has shown that it is probably a single ascospore culture. The significance of this fact will be discussed in later papers.

Plate 2, figs. d, e, and f show the colonies appearing when the D strain is sown for the first time on MDY, CM, and Pr agar. This strain yields relatively higher than the R strain but has less baking strength, i.e., it requires longer to cause bread to rise. Genetic analysis has shown these two strains to be distinctly different. It is apparent that this genetic difference results in a larger number of D strain cells surviving the transfer. In this strain there are also many fewer variations in colony size on both the CM and Pr agars, which is in line with the view that the variations in colony size are proportional to the severity to the shock of transfer.

SUMMARY

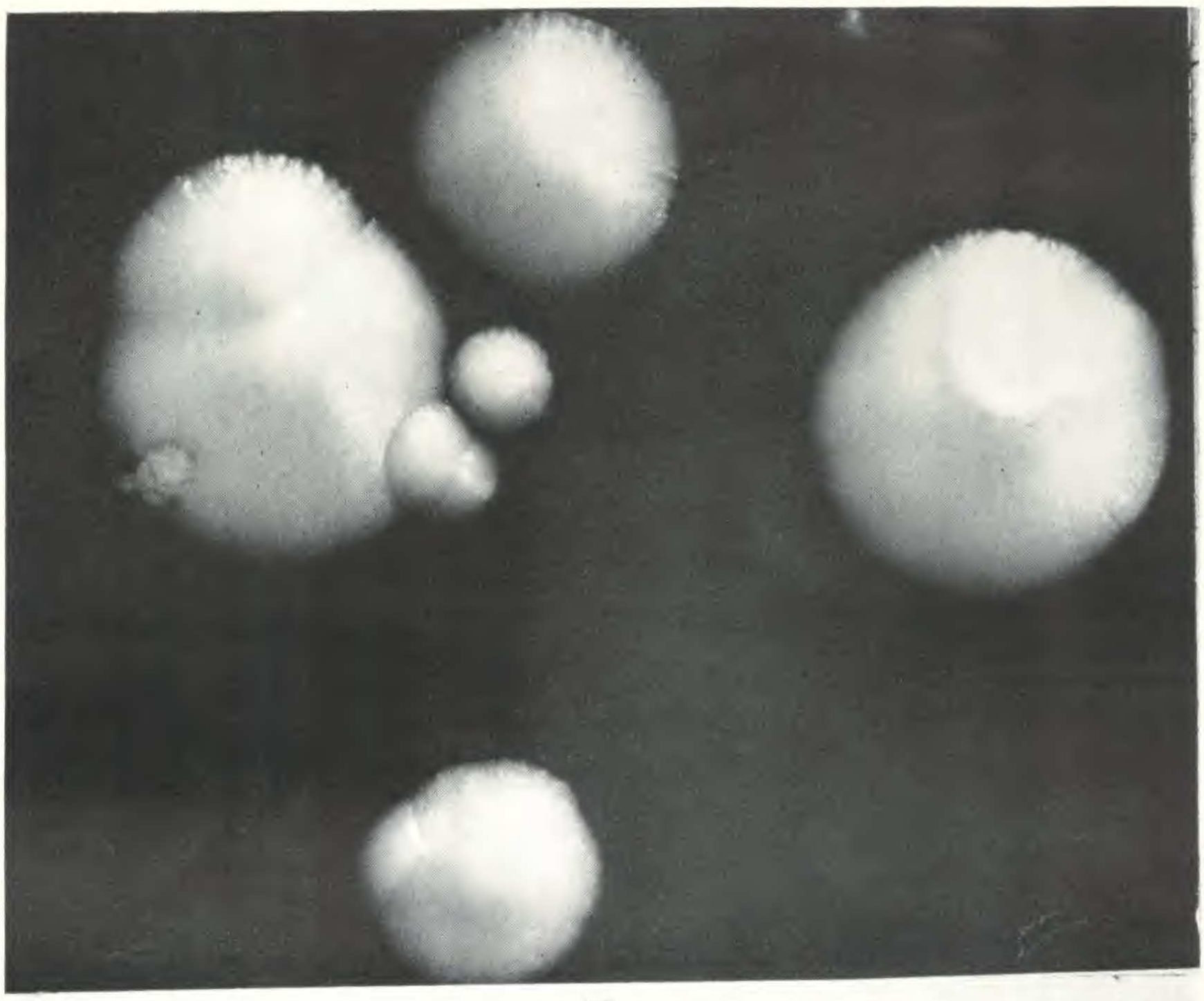
A test which indicates the efficiency of a yeast in transforming nutrient materials into yeast cells has been developed and its reliability studied. Many commercial yeasts were found to produce two classes of colonies when planted on a good medium. We have called the large smooth colonies, primary colonies, and the small variable colonies, secondary colonies. The secondary colonies are low yielders. The distinction between primary and secondary colonies fails when yeasts are planted on poor media because the shock of transfer causes great variation in colony size.

Explanation of Plate

PLATE 2

Fig. a. Typical large and smooth primary colonies with various small and rough secondary variants on MDY agar.

Fig. b. Large hemispherical white primary colonies and flat translucent gray low-yielding secondary colonies plated from a cake of commercial yeast.



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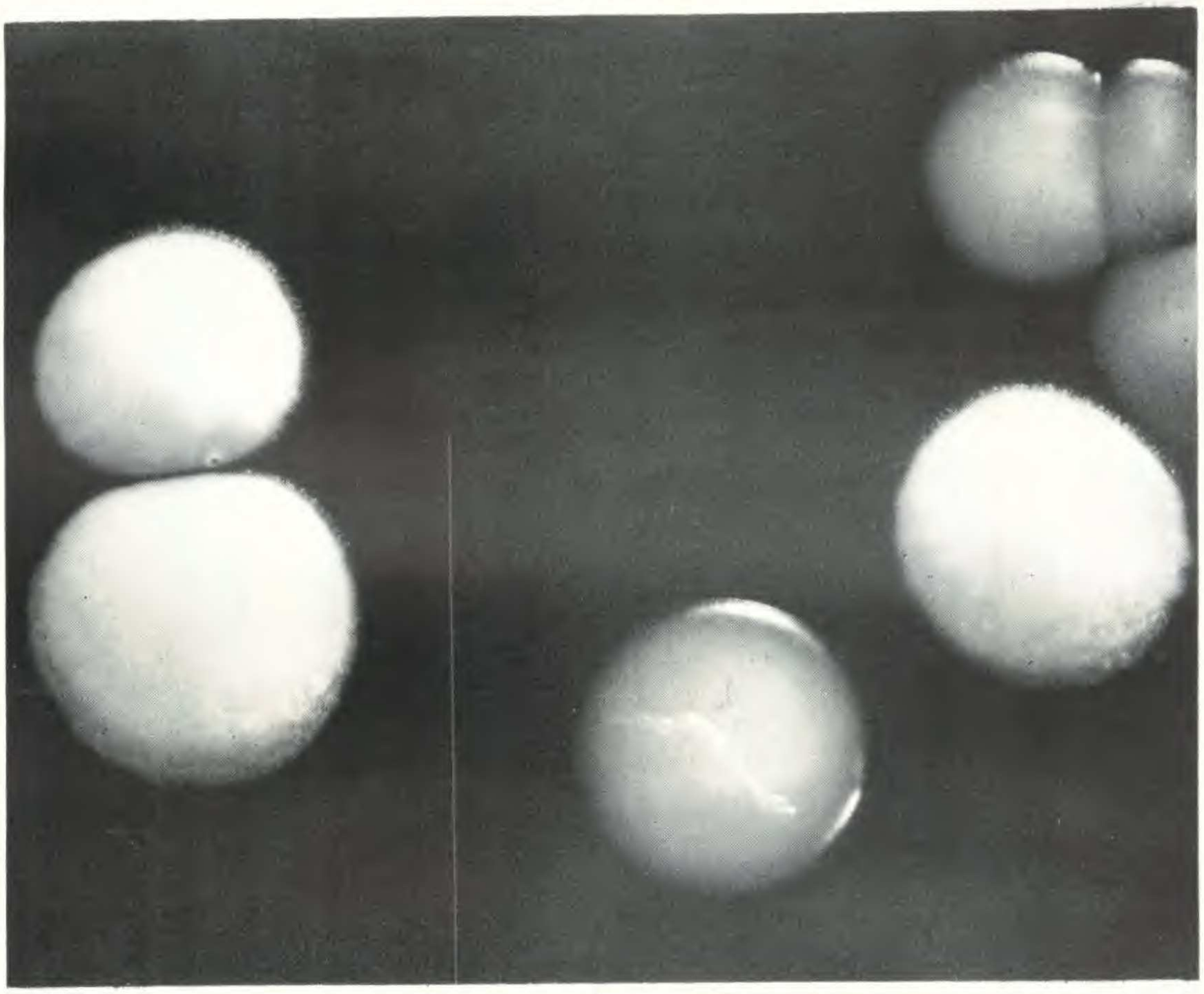

b

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EXPLANATION OF PLATE

PLATE 3

Figs. a, b and c. Colonies of the R strain of yeast appearing after plating equal number of cells on MDY (a), CM (b) and Pr (c) agars. Two true secondary colonies appear on the MDY plate. On the CM plate there is appearing a great reduction in the number of colonies and much variation in size. On this plate variations in size bear no relation to yield.

Figs. d, e and f. Colonies of the D strain of yeast appearing on MDY (d), CM (e) and Pr (f) agars. This yeast is genetically different from the R strains and withstands the shock of transfer much more successfully.